SOME FURTHER RECORDS CONCERNING THE PHYSIOLOGY OF REGENERATION IN TUBULARIA.

T. H. MORGAN.

The following experiments are in the main continuations of previous ones carried out to test further certain results whose interpretation was in doubt owing to insufficient data. The main problem concerned the influence of regeneration at the oral end on the rate of regeneration at the basal end. Experience shows that caution must be used in interpreting the experiments that bear on this point, because, in the first place, the rate of regeneration is greatly influenced by slight changes in temperature, and therefore accurate results along these lines should be carried out in the future in water kept at a uniform temperature. The difference of temperature of day and night is a serious obstacle when dishes are kept at room temperature. In the second place the aeration of the water is also an important factor and should be controlled. The extent of surface exposure of the water, the number of pieces in a dish, *and the number of other organisms attached to the stems*, also influence the time of regeneration, in the latter cases by utilizing the oxygen or by fouling the water. In the third place there can be little doubt that an internal "condition" of the colonies plays a rôle in the result. Control pieces from the same colony should always be present, but even this precaution is not entirely sufficient, since different stems of the same colony may differ in their internal conditions.

Despite these difficulties some general conclusions may be drawn, although certain of the experiments will be more profitable when the external conditions are more fully controlled. By keeping the dishes surrounded by running water I have met some of these difficulties in many of the experiments where rate of regeneration is involved.

The Simultaneous Development of Oral and Basal Polyps.

As previous work has shown, the "rule" for pieces of the stem of *Tubularia* is for the oral primordium of the polyp to develop first, and then, after several days for the basal polyp to develop unless stolons have already appeared; yet not infrequently the oral and basal ends develop simultaneously. In a few instances the basal hydranths may appear before the oral. It was first shown by Driesch and afterwards confirmed by myself that the time of appearance of the oral hydranth depends on its distance from the oral end. The same relation is observed for basal polyps also. It is therefore of some interest to compare the time of regeneration of oral and basal polyps in those cases where both polyps appear simultaneously to see if any acceleration or retardation can be observed.

In one case after 50½ hours 23 of 75 pieces showed the beginning of oral primordia. There were amongst these, three "double" pieces, *i. e.*, pieces with both oral and basal primordia. In two of these the oral end was more developed; in one the basal end was ahead. After another 24 hours there were 43 oral primordia, of which 21 were on "double" pieces. There were also 3 pieces with only basal primordia. Thus at first there was an excess of oral development, but the number of basal primordia increased at the second observation. A large number of pieces had not produced as yet the oral primordia, and three of these pieces had basal primordia only. The results show that both ends may develop simultaneously, and in such cases the rate of development may not be behind that when only one end develops. It is evident therefore that, if the stimulus to develop is present, two polyps may develop at the same rate as does a single one.

In another case, after 45 hours, 56 of 92 pieces had oral primordia only. Twenty-four hours later there were 28 double pieces. In another case after 45 hours, 27 of 55 pieces had oral primordia only. Twenty-four hours later there were 27 oral primordia, and in addition 18 double pieces.

In another case, after 48 hours, 10 of 29 pieces were double; of these 6 were equally developed at each end, 3 had the oral end ahead and 1 the basal. In another case at another time, after

24 hours 10 of 55 pieces showed only oral primordia. Twelve hours later 29 of 55 pieces had oral primordia, and of these 3 pieces were double — two of them having the basal end ahead.

Three other cases are summarized in the table.

48–50 Hours.

Oral.	Double.	Basal.	Nothing.
10	3		4
19	14	5	
9	27	4	

These facts show that despite the "rule," the number of double and basal polyps is considerable. Pieces similar to these must be present when the oral ends of pieces are tied. Consequently the rate of basal development of many of the tied pieces may have no connection with the tying of the oral end. Nevertheless comparisons with the control show unmistakably that the percentage of basal polyps is greatly increased by oral ligatures.

In order to see whether the development of the basal polyps in double pieces is accelerated when the basal and the oral ends develop simultaneously, pieces of moderate length were cut off from the distal end of stems and the basal continuations of these pieces were also kept and a record made of the time of their oral development.

In one case after 26½ hours there were faint indications of oral primordia on 15 of 34 of the distal pieces. At this time 5 of the basal pieces also showed oral primordia. Seven hours later the former pieces had 21 oral, the latter 6 oral primordia. Fifteen hours later the former had 29 oral, the latter 15 oral primordia or polyps and one double primordium. The same proportions were found 48 hours later. In this case no double pieces developed at first among the distal pieces, although the proximal pieces early showed primordia. This negative result shows that the basal development of double pieces is not necessarily correlated with the oral development of their basal partners.

In another case, of 50 distal pieces, after 26½ hours, 14 had oral primordia, the 25 basal pieces had 10 oral primordia. Seven hours later the former had 25, the latter 18 oral primordia. Twenty-seven hours later still the former had 32 oral primordia and one basal primordium, the latter 23 oral and one double. Later the same relations held.

In a third case, after 24 hours, 6 of the distal pieces had oral primordia, and none of the basal pieces had developed. Twenty-four hours later the former had 32 oral primordia and one basal. Some of the basal pieces had developed. Twenty-four hours later still the former had 33 oral, 3 double and one basal primordium ; the latter 8 oral.

In a fourth case, after 48 hours, the distal pieces had 16 oral and two double premordia ; the basal pieces showed 11 oral, 4 double and 6 nothing.

In a fifth case, after 48 hours, the distal pieces had 28 oral and 13 double primordia; the basal pieces 12 oral and one double primordium.

The results seem to show no acceleration owing to oral development, and on the other hand no necessary retardation if both primordia start at the same time. Local factors must therefore determine the results. Most of the distal pieces served also as controls in cases in which the oral ends of other pieces had been tied. In all such cases there was a very great excess of basal primordia in the orally-tied pieces, showing that there is a distinct effect produced in this way. It would seem therefore that despite the fact that both oral and basal polyps may develop simultaneously, the oral development, if it gets the start, inhibits to some extent the *beginning* of basal development. The nature of this influence is still obscure.

Are the Changes in Isolated Pieces Local or General?

Pieces were tied near the oral end and then after a number of hours as much of the basal end as would contain the primordium of the polyp was cut off. In the first experiment the basal cut was made ten hours after tying. Twenty-three hours after the beginning 3 of 9 pieces showed basal primordia. In the control (tied but not cut) 3 of 9 pieces also showed basal primordia. The removal of the basal piece had not, within a total of twenty-three hours, delayed the basal development. In other words the cut end did in thirteen hours what it took the control twenty-three hours to do. This means that the rate of regeneration was accelerated by changes taking place in the pieces at least some little distance from the cut end. No doubt the development would have

been delayed if a larger basal piece had been cut off, but unfortunately no experiments were made to show this. It is however probable that the changes are especially confined to the basal region near the cut end and are less at more distant points.

The converse experiment consisted in cutting off pieces of the oral end. The basal end was tied in the first experiments, although this is not necessary, because tying the basal end does not accelerate the oral development.

When the oral ends were cut off after ten hours the six pieces showed, 23½ hours after the beginning, 3 faint oral primordia. The 6 controls had all oral primordia. After 72 hours all the 6 cut-off pieces had oral polyps, while only two of the controls had oral polyps and two oral primordia. The cut-off pieces appear to have fully caught up with the controls. The data are indeed very few, but the results confirm earlier ones thus obtained. In order to see whether when both ends of a piece are closed any processes take place that accelerate development if the ends are later exposed, the following experiment was made. Both basal and oral ends were tied and later the piece was cut in two in the middle. In order to ascertain the rate for normal regeneration control pieces were cut off and left open. These developed oral primordia after 19 hours in 9 of 14 pieces. These pieces were then also cut in two in the middle. After 55 hours (from the time of tying) the basal halves of the tied and cut set produced 5 oral primordia, while of the control 6 had oral primordia, 2 basal primordia, and one was a double piece. The results indicate that when a piece is closed at both ends no changes take place in it that accelerate the development in the middle of the piece. The result may throw some light on the preceding experiment, and if so, shows that the changes that there took place were due to the open end.

Is Basal Development Accelerated by Allowing the Oral End to Begin its Development?

In one of my previous papers[1] I gave the results of an experiment in which pieces were tied near the oral end after having been left open for several hours. The experiment was made in

[1] *Journ. Exp. Zoölogy*, II., 1905.

order to see if the amount of materials set free by the breaking down of the ridges near the oral end (preparatory to the development there of a polyp) has an influence on the rate of basal development. As a control some pieces were also tied at once at the same level. One such experiment is shown in the next table.

AFTER 48 HOURS.

Control.	Tied after 6 hours.	Tied after 20 hours.
11 Basal	8 Basal	3 Basal
2 Nothing		

Another experiment gave similar results. In a third experiment pieces, tied near the oral end after 9 hours, produced basal primordia before those tied at once.

In this third experiment there was perhaps some indication that when pieces were left open for a time at both ends and were then tied at the oral end the basal polyps developed sooner than when the oral ends were tied at once. This result I had obtained before. The three following experiments do not give the same result.

Of 12 pieces tied at once at the oral end, 2 had basal primordia after 48 hours. Of 11 pieces tied after 26½ hours 3 had basal primordia. After another 24 hours the former had 5 primordia and 6 polyps, the latter 10 basal primordia (and one nothing). These were less advanced than those tied at once.

In another experiment 16 pieces were tied at once near the oral end and produced after 48 hours 14 basal primordia. Of 8 pieces tied after 26 hours 6 had basal primordia. The evidence seems to show that no retardation occurs (or very little) if the oral ends are tied after 9–24 hours as compared with pieces tied at once, and there may be an actual, *i. e.*, an absolute acceleration.

The results of another experiment are also still doubtful after repetition of it. Pieces were left open for several hours and a ligature was then tied near the oral end in some of the pieces, and in others near the basal end. The time of development of the basal primordia was noted in each case. Previous experiments had given some indication that the longer basal pieces produced primordia before the shorter ones. It seemed not improbable, if this were really true, that the amount of material set free

from the oral end accelerated basal development, for more of it would be shut off in longer pieces, although not proportionally more. The new experiments showed that in some cases the pieces ligated nearer the basal end developed as soon as those ligated more orally. Moreover the ligature itself if too near the basal end may interfere with development there. It appears then that it is still unsafe to draw any conclusions from these experiments.

Stolon Formation.

Stolons develop after several days in a small percentage of cases from the basal ends of pieces lying on their side in the dish. More often the basal ends produce heteromorphic polyps. The absence of stolons from the oral ends was first noted by Loeb. The potentiality to produce stolons must of course be present throughout the piece, since they may form from basal cut ends at any level. I attempted in the following way to produce them at the oral ends by first tying pieces so that basal polyps or primordia appeared, and then by cutting off the piece behind the ligature. The presence of the basal polyp might be imagined to make the conditions favorable for the development of oral stolons. This would certainly be the expectation if the polarity of the whole piece is reversed as Loeb supposed. In no case were oral stolons produced in this way, even when the pieces with basal polyps were cut off near to the polyps.

In one case the pieces were cut off below the ligature 10 hours after tying. After 48 hours from the beginning there were 3 oral primordia and no basal. Other pieces had been cut off after 24 hours (when the ligatured controls showed 10 of 27 pieces with basal primordia). All 14 pieces showed oral primordia, and 8 of these had primordia also at the basal end more developed than those at the oral end. The presence of basal primordia had not delayed the development of oral primordia. In fact the latter appear to have been accelerated — not however necessarily owing to the basal development but to other changes in the piece.

It is probable that external factors combined with the internal factor called polarity has something to do with stolon-formation. But even when the oral ends of pieces with basal polyps were

covered with sand or were thrust into little heaps of sand no oral stolons developed.

Influence of Diluted Sea Water.

Some observations of C. D. Snyder on *Tubularia crocea* of the Pacific Coast seemed to him to indicate that in dilute sea water more basal polyps are produced than in normal sea water. In several experiments that I made with *T. crocea* of Woods Hole in which the sea water was diluted no more basal polyps developed than in the control in sea water. The occurrence of basal polyps is so variable that only a thoroughly controlled set of experiments would suffice to prove that dilution affects basal polyp formation.

Direction of Currents.

In *Tubularia* a current passes up one side of the hollow stem and down the opposite side. When more than one dissepiment is present there may be two currents in one direction and one or two in the reverse direction. Those who maintain (Goebel and Loeb, for example) that currents may determine polarity might hope to find in the direction of these currents in *Tubularia* a solution of the problem of polarity. In fact Loeb has suggested such an hypothesis. Since however there is a current in each direction it is difficult to understand how this idea could be supposed to account for the difference in the behavior of the two ends, unless indeed there is a dorsal and a ventral side to *Tubularia* (which seems to be radially symmetrical) so that the dorsal side of the oral polyp is on the opposite side from that of the basal polyp. This supposition is not however in harmony with the heteromorphosis in other forms, such as the earthworm or planarians. In these the ventral and dorsal surfaces are the same in the old and in the heteromorphic structures. The only alternative would be to expect, if the currents in Tubularia are a factor in the direction of regeneration, to find that the current near the basal, or heteromorphic polyp is reversed in direction as compared with that going to the oral polyp.

I have examined the direction of the current in a number of long double pieces, and have found that the direction is continuous along each half of the piece and not different at the two ends.

This observation sets aside, I think, any attempt to explain polyp-formation on the physiological basis of the direction of the currents. The inadequacy of such an explanation is also readily seen when short pieces with "double" partial hydranths are examined, in which the continuous current is readily demonstrated.

"Polarity."

In an earlier paper I have pointed out that experiments show that there exists a graduation in the materials of the stem of *Tubularia*, and that on this as a basis we may attempt to adjust our conception of polarity. This gradation can be seen structurally in the change in the thickness of the walls and in the histological character of the cells of different levels. Physiologically it is shown in the more rapid regeneration of the cut surfaces the nearer they are to the distal end. This difference in rate I supposed might be due to the greater amount of "hydranth-forming" materials[1] near the distal end, or more correctly to the less differentiation of the more distal parts. It was perhaps unfortunate to have used the words hydranth-forming materials, for it might readily be inferred that I meant to refer to formative materials or substances as such, *i. e.*, independently of the differentiation that decreases in amount distally. A careful perusal of the text, especially of later papers, will show that I had not so much in view the presence or absence of peculiar and unknown "stuffs" (a view I have often disputed) as the direction that differentiation had taken in different regions. The more distal parts of the stem are less specialized as storage and supporting tissues than the basal parts. The distal region, having as it were, less to undo, develops more quickly into a polyp. This sort of difference of materials, with its concomitant physiological differences, may furnish a basis for that particular condition

[1] By hydranth-forming materials I meant not reserve stuffs or "organ-forming substances" in the sense in which some embryologists of the preformation school use these terms, but rather materials that have already been differentiated into a part of the anterior end of the body or head. The phrase is more applicable to cases where the head is not sharply marked off from the trunk as in the earthworm, in planarians, etc. That the direction in which differentiation of a tissue has taken place is an important factor in determining the future course of regeneration of that tissue is familiar. By generalizing this fact I have attempted to make it the basis for the phenomena of polarity.

that we call polarity. These material differences with their correlated physiological differences lead to differences in the behavior of the two ends, for, while the material exposed at any cross-cut is the same at both ends, it behaves differently on account of the relation of the end to neighboring regions. This relation may be thought of as one of direction and this is polarity.

It has seemed to me possible that the relation of the parts to each other might be expressed as the relative condition of tension — in a physiological sense — that exists between the different parts. Further analysis has led me to think that behind this relation there is a more subtle one and that irritability is the physiological factor that regulates the behavior of the cells in development and in regeneration. Even the differentiation of the different regions must be supposed to be due to their relation to neighboring regions. In fact, one of the first and most obvious changes that takes place in cells when removed from contact with their fellows is a loss of differentiation, followed by a re-differentiation in relation to a new terminal condition. Polarity therefore in the last analysis stands for a graded relation resting on a condition of contractility (tension) that exists between different levels.

The Cause of the Delay in Basal Polyp-formation when the Oral Polyp Develops First.

In my last two papers on Tubularia I have laid perhaps undue emphasis on the question as to how the basal development of a piece is accelerated when the oral end is tied and conversely as to how the basal end is retarded when the oral end develops. The problem interested me because of its apparent wider bearings; for it seemed possible in this case to test by suitable experiments whether the result could be explained on purely chemical grounds or whether a different principle was involved. The attempt to find a sufficient chemical stimulus does not appear to me to have been successful, for the confessedly incomplete data on which my conclusion provisionally rested has not withstood a second attack. It seems to me now not improbable — and more can not at present be said — that the retardation of the basal development is directly owing to the formative changes taking place at the oral end.

The simultaneous development of oral and basal polyps that sometimes takes place indicates that there need be no absolute antagonism between the development of polyps at opposite ends, but only that such an influence tends to inhibit the beginning stage of the polyp. It is also significant, I think, that the basal development has a smaller retarding influence on the oral development than vice versa, as the experiments show. This means apparently that the locally stimulating factors have a stronger influence on an oral end, due possibly to the direction of the gradation in the pieces. Other experiments show that this direction is a factor in determining the rate. It may seem that this view could be tested by a comparison of pieces of different lengths, for a greater influence would be anticipated in shorter pieces, but it is difficult to make such a test since the decrease in the rate of response when the cut lies nearer the base seriously interferes with a fair comparison being made.

The Factors Involved in the Closure of the Open, Cut Ends.

The extremely rapid closure of the cut end of a stem by means of a plate of cells that advances diaphragm-like from the cut edge, has always excited my interest, because it throws a good deal of light on the way in which movements of materials may take place without any proliferation of new material entering into the process.

Renewed study has shown that the oral and the basal ends of pieces close at the same rate; that the edges of oral, cut surfaces from the distal region of the stems close approximately at the same rate as oral surfaces near the basal regions; that the time of complete closure depends on the size of the piece, smaller pieces closing sooner, since the rate of advance of the edge is about the same in all; and that the addition of salts and of sugars to the sea water, if not so great in amount as to involve serious changes, affect very little the rate of closure. Surface tension therefore seems inadequate to explain the results, although surface tension may play a minor rôle as a part of the stimulus to contraction.

Sections and surface mounts of different stages in the process of

closure, disclose several points of interest. The membrane, that forms and advances ring-like toward the center, is relatively thick and is composed of a large number of cells. The slight withdrawal of the cœnosarc from the cut end is insufficient to account for the presence of so many cells, and the only interpretation that remains is that the cells of both ecto- and endoderm must be drawn towards the cut surface for some little distance from the ends, although the closure of quite short pieces shows that the result may also be attained by a shorter length being utilized. This involves a greater decrease in thickness of the neighboring cœnosarc wall.

What factors are involved in the closure? We may suppose that the stimulus of the sea water, or surface tension, or the loss of contact relations of the material, with its concomitant change of tension relations, is the initiatory stimulus. The last seems to me the main factor for reasons given in previous papers. The tension equilibrium lost, contraction follows. The cut edge contracts as a whole, and as a result of the intimate fusion of the outer layer of the ectoderm with the perisarc the diaphragm-like membrane develops; for if separation from the perisarc is first accomplished a different kind of closure takes place. As the process continues the cells are drawn out bodily over the transversely closing membrane. Despite the rather intimate union of the cells with each other they not only change shape but shift their relations to each other, and while at first a considerable number of cells reach the edge of the closing membrane, their number becomes fewer as the opening gets smaller.

Our analysis leads to the conclusion that the closure is a contractile process of the living substance.

When closure is completed the relation of the parts to each other is still an unstable one for the end of a piece. This leads to further changes in conformity with the irritable nature of the materials and a new polyp develops in consequence. If our analysis is correct we are led to the view that the essential factor in the closure of the stem is the irritability of the protoplasm which leads it to undergo movements (through its power of contractility) of a peculiar kind. This same process, that we find reduced to its simplest term in the closure of the stem, is the mainspring

also of all or most of the formative changes that follow. Irritability therefore is the essential factor in formative action and leads first to change in position of the cells and ultimately to their differentiation. Yet the process of closing gives every appearance of taking place independently of the cells as units ; for the movements *appear* to take place in the material as a whole and not to be the sum total of a vast array of cell-adjustments. In other words the cells adapt themselves as best they can to the mass action that is going on and do not take the initiative in the process. Nevertheless it is unsafe to argue from such gross effects that cells have no independent functions. Their power to divide at different times, and the cellular limits of differentiation, show their independence in certain respects. If, however, as seems probable, the cells are united by a meshwork of protoplasm the irritability of the living material may act without heed to cell boundaries.

The Nature of Formative Action.

After the closure of the end of the stem of *Tubularia* the condition is still one of unstable equilibrium ; by which I mean that the stimuli received at the closed end cause further changes in the relation of the end to the rest of the piece. The stimuli may be entirely or largely internal, resulting from an unstable termination. In *Tubularia* both external and internal factors may act. This relation leads to the formation of a new polyp in which the relation of the materials to the outside world and to the rest of the piece is a stable one. If my analysis of the factors involved, first in the closure of the stem, and later in its further elaboration into a polyp, is correct we are led to the view that the essential factor is the irritability of the material that determines the location of the formative changes. As I have given recently (the Seventh International Congress, August, 1906) my reasons for coming to this general conclusion I shall not enter further into the argument here, but desire only to bring the interpretation into connection with the present results.

The modern school of developmental mechanics has sought to explain formative actions as the result of familiar chemical and physical phenomena but has not met with any marked

success in explaining such actions. The vitalistic view goes to the opposite extreme and postulates unknown or unknowable processes. Both seem to have overlooked the possibility of accounting for formative changes by the familiar processes of irritability and contractility, that appear to be fundamental attributes of living materials. These cannot be reduced as yet to any known chemical or physical phenomena, but neither does it follow that they belong to a vitalistic category.

To sum up: the formative principle as seen in development, growth, and regeneration, appears to be an expression of the irritability of the living material. The problem of formative action is therefore intrinsically one of response to external stimuli or to internal relations between the parts through the agency of irritability. Contractility is one of the most usual methods of responding to the condition of stimulation that exists in a given region, while differentiation follows quickly in its train. The formative principle is the outcome of a factor that is not one of the familiar chemical or physical events.[1] This conclusion does not force us into vitalism; for until we know something of the nature of irritability it is premature to insist on referring it to any larger category. As yet we are only at the threshold of a knowledge of formative changes.

[1] To those who refer the varied manifestations of irritability to a psychical principle the problem of development will appear to belong to that category; but to those who see in irritability only a "physiological process," the same problem will seem to be physiological. The distinction may be verbal and conventional.